Research Article | Vol. 10, No. 7 | 1 Jul 2019 | BIOMEDICAL OPTICS EXPRESS 3232
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Active Er-laser drug delivery using drug-impregnated gel for treatment of nail diseases

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Abstract: Active Er-laser drug delivery under the nail plate using a drug-impregnated gel containing liquid methylene blue clusters is demonstrated for the first time. The effect of the agar-agar concentration in the gel and the gel plate thickness on the number of Er:YLF-laser pulses required for formation of a through microhole in the gel and in the nail plate with subsequent active drug delivery is discussed. The influence of the laser pulse energy, the gel plate thickness, and the external pressure applied to the gel on the rate of delivery of methylene blue under the nail plate through a single microhole in it is investigated. It is shown that with a laser pulse energy of 4.0 ± 0.1 mJ, the delivery rate can reach 0.024 ± 0.004 mg/pulse.

1. Introduction

Currently, onychomycosis (fungal infection) and psoriasis of the nails are widespread diseases [1], and the search for effective methods of their treatment remains an important problem in dermatology. Treatment options for nail diseases include the use of systemic and local drugs or their combinations. Systemic therapy has many drawbacks: high toxicity, a wide range of contraindications, high probability of interaction with other drugs, a significant duration of therapy and a high frequency of relapses [2]. Local therapy of nail diseases is limited by the low permeability of the nail plate for drugs due to the high density of keratinocytes [1].

The permeability of the nail plate for drugs can be increased using chemical (acids, alcohols, glycols, etc.), physical (microperforation, iontophoresis, sonophoresis, electroporation) and mechanical (injection, removal of the nail plate) methods [3–14]. The nail permeability can be improved by using permeation enhances such as methanol and dimethyl sulfoxide. In this case, the permeation coefficient reaches 5-7.5 × 10^{-8} cm/s, but still remains extremely low [13]. Microperforation of the nail plate facilitates the penetration of therapeutic dose of the drug into the deep layers of the nail plate and into the nail bed to eliminate the source of nail diseases and to accelerate the process of nail diseases treatment [14]. Microperforation of the nail can be carried out by various methods, including using a laser radiation [14–18]. The possibility of using the radiation of Er:YAG (λ = 2.94 μm) [14–16], Ti:Sapphire (λ = 1.053 μm) [16], XeCl (λ = 0.308 μm) [16], Nd:YAG (λ = 1.064 μm) [16], CO_2 (λ = 10.6 μm) [15,17], Ho:YSGG (λ = 2.08 μm) [6], Yb,Er:Glass (λ = 1.54 μm) [18], and Er:YLF (λ = 2.81 μm) [18] lasers for microperforation of the human nail plate was investigated. The highest ablation rate of the nail plate was observed when exposed to Er:YAG or Er:YLF-laser radiation.

Drug delivery through a microperforated nail plate to the infected nail bed can be passive or active, i.e. occur without or as a result of external action. In passive delivery, water-based drugs do not penetrate through microperforated nail to the nail bed due to the high surface tension coefficient, and alcohol-based drugs penetrate very slow [19]. Methods of active drug delivery through the microperforated nail plate have not yet been discussed in the literature.

#361343 https://doi.org/10.1364/BOE.10.003232
Journal © 2019 Received 4 Mar 2019; revised 28 May 2019; accepted 29 May 2019; published 12 Jun 2019

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However, it is known that in active delivery the penetration rate of microparticles and liquids into soft tissues (skin) increases significantly as a result of Er-laser-induced hydrodynamic shock waves [20]. The paper [21] considered the finger tissue model, which allowed to describe the penetration of light into the tissue of the nail. The methods of active laser drug delivery are described in sufficient detail in [22–24], including the delivery of drugs originally in the form of a liquid or placed in special devices (films, cuvettes, etc.) [23]. The impact of an Er-laser pulse on a liquid drug causes a temperature rise, which can lead to evaporation and the formation of a steam-gas bubbles [24,25]. In this case, both the recoil pressure pulses at the evaporation front and the fluid flows resulting from the collapse of the bubbles can contribute to the drug delivery through a microhole in the nail plate.

It can be noted that the use of drugs in liquid form is associated with their spreading and splashing, the patient and the doctor can easily get dirty in the process of applying the liquid drug. In addition, control of the thickness of the drug layer applied to the surface of a biological tissue is a non-trivial task and requires the use of very expensive equipment [26]. Therefore, it is promising to use a drug-impregnated gel devoid of the above disadvantages. Biopolymers (alginates, collagen, gelatin, agar-agar, chitosan, hyaluronic acid, polyethers of bacterial origin) can be used to create implants, for the prevention of the formation of coarse postoperative scar tissue in patients, for applying a gel coating in the treatment of various diseases, in the development of prolonged drug delivery systems, for photodynamic therapy (PDT) [27], etc. At the present time, the following types of gels are used for PDT: “Morion” gel (AREV PHARM LLC, Russia) with a solution of metal complexes of chlorin E6 derivatives [28], 1% Photoditazine gel [29], 1% Radachlorin gel [30], etc. Extraction of the active agent from the gel can occur spontaneously or as a result of external action, including mechanical extrusion.

Attention should be paid to the fact that there is no description of research results in the literature, in which the radiation of one and the same laser was used both for microperforation of the nail plate with a gel located on its surface and for active delivery of a drug contained in this gel.

In our study, we used the original agar-agar gel containing methylene blue (MB) clusters. Methylene blue is a widespread photosensitizer for use in photodynamic therapy of fungal diseases; it is non-toxic and has no side effects [31]. Agar-agar was chosen as a matrix for MB, as it is stable, biocompatible and can be easily prepared in laboratory. In addition, it promotes the absorption of inflammatory exudate, provides anesthetic and anti-inflammatory effect [32].

The purpose of this work is to determine the possibility of active Er:YLF-laser delivery of the drug (MB) through a single microhole created by radiation of the same laser in the nail plate and in a plate of a MB-containing gel placed on nail plate surface; to study the effect of agar-agar concentration in the gel and the thickness of the gel plate on the number of Er:YLF-laser pulses required for through microperforation of the gel plate, microperforation of the nail plate *in vitro* with a gel plate placed on its surface and subsequent active delivery; study of the influence of the laser pulse energy, the gel plate thickness and the external pressure applied to the gel for the extraction of MB from it on the rate of drug delivery through a single microhole in the nail plate *in vitro*.

2. Materials and methods

The scheme of the experimental setup is shown in Fig. 1(a). The radiation of Er:YLF laser (1) with a wavelength of $\lambda = 2.81$ μm passing through the focusing system (2) hit the surface of the gel plate (3) which was placed on the outer surface of the nail plate sample (4). The nail plate sample, in turn, was placed on a paper substrate (5), and the substrate was placed on the surface of a glass slide (6). From the back of the paper a digital USB-microscope (7) was installed connected to a computer (8).

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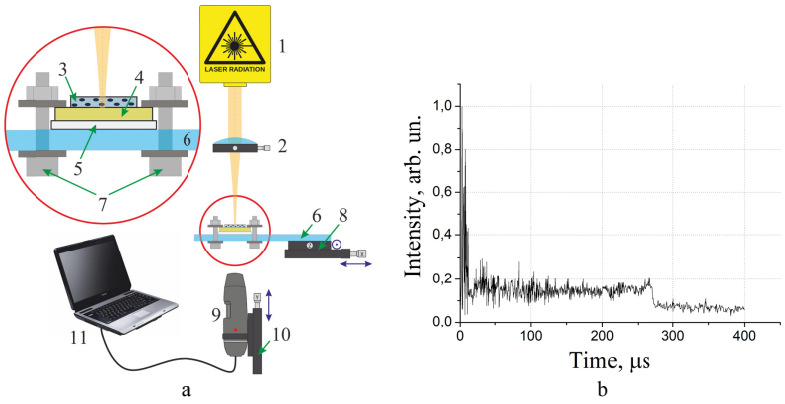

Fig. 1. Scheme of the experimental setup (a): 1 – Er:YLF-laser; 2 – collecting lens (F = 50 mm), 3 – gel plate; 4 –nail plate sample; 5 – paper substrate; 6 – glass slide; 7 – fastening screws; 8 – XZ-translator; 9 – digital USB-microscope "DTX 50" (Levenhuk, Inc., USA); 10 – Z-translator; 11 – computer; oscillogram of Er:YLF-laser radiation pulse (E = 4 mJ) (b).

Fragments of healthy human nail plates *in vitro* were used as samples for the study. The fragments were mechanically removed areas of the free edge of the nail. After extraction, the samples were stored at room temperature in a dry, dark place for no more than one week. Immediately before the experiment, the samples were mechanically cleaned of dirt and washed in a stream of distilled water for 1-2 minutes. A total of 80 samples were used in the study. The average sample thickness was 350 ± 10 μm. Of course, when treating a nail, the laser parameters for microperforation of affected by onychomycosis or psoriasis nail plate and providing active delivery can be different from the parameters necessary for a healthy nail treatment. This may be due to the fact that nail damage is usually accompanied by an increase in its thickness and dehydration of the nail plate [33]. Despite this the study of the microperforation of a healthy nail plate and subsequent active drug delivery is necessary, since the treatment of diseased organ is always performed within healthy tissues [34].

In the experiments, the laser pulse energy (E) was varied in the range of 1.0 ÷ 4.0 mJ by changing the diode pump power, the pulse repetition rate was f = 30 Hz. The duration of the Er:YLF-laser pulse was 270 ± 10 μs (base) (Fig. 1(b)). For microperforation, the energy E = 4.0 ± 0.1 mJ was used, at which microperforation of the nail plate occurred with the highest efficiency for this laser [18]. Laser radiation in all experiments was focused on the nail plate surface, and the spot size in the focal plane was 220 ± 15 μm.

To prepare the gel with concentrations of 0.5%, 1%, 2%, 3%, we added in an 0.25% aqueous solution of MB (250 mg of powder per 100 ml of distilled water) agar-agar powder (0.5, 1, 2, and 3 g of powder per 100 ml of MB, respectively). After that, the resulting solution with constant stirring was brought to a boil and boiled for two minutes. Then the solution was poured into a glass container and stored at room temperature in a dark place for 10 minutes, after which the gel block formed was mechanically removed from the container. The gel plates were cut from the block using razor blade "Gillette Platinum Plus" (P&G, USA) under the microscope. The edges of the gel plate were cut so that it covered the entire surface area of the nail plate sample and placed on the surface of the nail sample as shown in Fig. 1. The control of the gel plate thickness was carried out using an optical microscope "Axio Scope A1" with "Axio Cam Icm1" (Carl Zeiss, Germany) camera. The photos of the side slice of the gel plate were analyzed in the program "ZEN Lite" (Carl Zeiss, Germany).

As a result of the preparation procedure described above, we obtained a gel matrix containing clusters with a solution of methylene blue. Figure 2 shows micrographs of gel samples with different concentrations of agar-agar powder.

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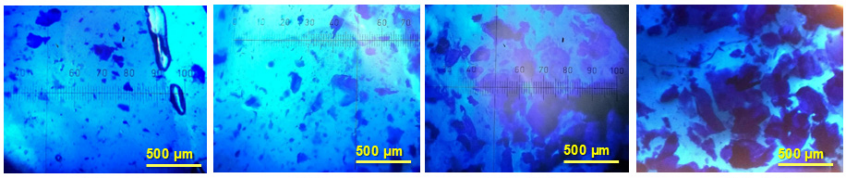

Fig. 2. Photos of the MB-containing gel with 0.5%, 1%, 2% and 3% concentration of agar-agar (transmitted light).

It can be seen that the sizes of the clusters with MB solution in the gel samples differ and are ~110 ± 40 µm, 180 ± 50 µm, 290 ± 50 µm and 350 ± 60 µm at 0.5%, 1%, 2%, and 3% gel concentration, respectively. Thus, varying the concentration of agar-agar, it is possible to change the size of the clusters with MB solution. The cluster size can be changed in a fairly wide range.

Under the action of laser radiation, a single microperforation was formed in the gel plate and then in the sample of the nail plate. A microhole in the nail plate was created through a layer of gel with a thickness of 100 ÷ 800 µm. To control the shape and depth of microholes, an optical-microscopic registration of the appearance of laser-produced microdamages in the gel and nail plate and the appearance of their longitudinal cuts (thin sections) was carried out using the "Axio Scope A1"("Carl Zeiss", Germany) optical microscope. The experiment determined the number of laser pulses required for through microperforation of a gel plate (N_{gp}) with different thickness (h_g) and agar-agar concentration (C_{aa}), as well as the number of laser pulses required for through microperforation of the nail plate (N_{np}) with the gel plate located on its surface. The number of pulses required for through microperforation of the gel plate (N_{gp}) was determined by the appearance of a hole on the back of the gel. When determining N_{gp}, the gel plate was placed on a glass substrate separately from the nail plate. Up to ten N_{gp} measurements were performed for each thickness and concentration of the gel. Next, the number of laser pulses required for through microperforation of the nail plate (N_{np}) was determined. When determining N_{np}, the laser impact was implemented on the nail plate with the gel placed on its surface. Knowing N_{gp}, we established N_{np} as a number of pulses required for the appearance of a microhole on the back of the nail plate minus N_{gp}. Up to ten N_{np} measurements were performed for each thickness and concentration of the gel plate on ten fragments of nail plates. The same way, knowing of N_{gp} и N_{np}, the number of laser pulses (N_{ad}) required for active delivery of MB was determined. In this case, staining of the paper substrate placed under the nail plate indicated the drug delivery. Up to ten N_{ad} measurements were performed for each thickness and concentration of the gel plate, as well as external pressure on seventeen fragments of nail plates. To establish all of the above events (the fact of microperforation of the gel plate, the nail plate and the fact of MB penetration) we used digital USB-microscope "DTX 50" (Levenhuk, Inc., USA).

To extract the MB from the gel clusters into a microhole formed by laser radiation, an external mechanical action was applied to the gel plate for 3.0 s – a load of known mass was placed on the gel plate. The external mechanical action was made by a cylindrical stainless-steel object with diameter of 2 ± 0.1 mm, which was placed on the gel plate 5 ± 1 s after its microperforation. The mass of the load changed from sample to sample, and the size of the contact patch did not change and was 2 ± 0.1 mm, which made it possible to change the pressure in the range P = 0.3 ÷ 2.0 kPa. As a result of this external action, the microhole in the gel was filled with methylene blue (the fact of filling was observed under a microscope), and the microperforation in the nail plate remained unfilled. It should be noted that without external action we did not observe filling of the microhole with the MB solution, wherein the maximum duration of the filling is limited to the drying time of the gel plate.

Immediately after the termination of the external mechanical action, the MB-filled microhole was irradiated with Er:YLF-laser pulses with an energy E = 1.0, 2.0, 3.0 or 4.0 mJ,

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as a result of which active delivery of methylene blue through the microhole in the nail plate was expected. To visualize the fact of delivery, we used paper with a density of 80 g/m and a thickness of 100 μm placed under the nail plate. When the MB penetrated through the microhole, the paper turned blue. The nail plate and paper were pressed against the glass slide in such a way as to ensure tight contact between them. The fact of MB penetration was recorded using a digital USB-microscope "DTX 50" (Levenhuk, Inc., USA), which was located under the glass slide (see Fig. 1). So, the fact of MB penetration ("initialization" of drug delivery) was indicated as staining of the paper over the entire depth. The laser was terminated immediately after the "initialization" of MB delivery.

The mass (*PM*) of methylene blue solution penetrated under the nail plate with a single microhole was determined by weighing a piece of paper before and after the "initialization" of drug delivery. The dose (D_{MB}) of MB penetrated under the nail plate was defined as the ratio of the mass penetrated during the "initialization" to the area of a single microhole in the nail plate. The rate (V_{MB}) of MB delivery through a single microhole was defined as the ratio of the mass *PM* to the number of pulses (N_{ad}) required for the active delivery of MB after the termination of external action on the gel plate.

Statistical processing of the data obtained in the experiments consisted in determining the mean values and standard deviation of the measured quantities and was performed in the software package "STATGRAPHICS Plus 5.0" (Statistical Graphics Corp., USA).

3. Results and discussion

Active laser delivery of MB using the gel includes three consecutive stages of laser exposure: gel microperforation (first stage), nail plate microperforation (second stage), and active laser drug delivery (third stage). The diameter of the microperforation in the gel was d_g = 250 ± 10 μm, and in the nail plate – d_{np} = 220 ± 10 μm. The measurement results of the number of laser pulses required for through microperforation of the gel plate (N_{gp}), the number of laser pulses required for through microperforation of the nail plate (N_{np}) and the number of laser pulses (N_{ad}) required for active delivery of MB after the external action on the gel plate (P = 1.3 kPa), and the range in which lies the total number (N_Σ) of Er:YLF-laser pulses with E = 4.0 ± 0.1 mJ required for the formation of microholes in the gel and nail plate followed by active drug delivery are shown in Table 1.

Table 1. Results of N_{gp}, N_{np}, N_{nd}, N_Σ measurements at different values of h_g and C_{aa} (E = 4.0 ± 0.1 mJ)

h_g, μm	N_{gp}				N_{np}	N_{ad}	N_Σ
	C_{aa} = 0.5%	C_{aa} = 1%	C_{aa} = 2%	C_{aa} = 3%			
100	90 ± 8	60 ± 4	32 ± 4	20 ± 2	10 ± 1	4 ± 1	34 ÷ 104
300	184 ± 10	75 ± 8	48 ± 5	24 ± 4	10 ± 1	4 ± 1	38 ÷ 198
500	210 ± 15	150 ± 10	60 ± 5	30 ± 4	10 ± 1	4 ± 1	44 ÷ 224
800	315 ± 22	180 ± 15	150 ± 12	90 ± 10	10 ± 1	8 ± 1	108 ÷ 333

In all cases, N_{np} = 10 ± 1 laser pulses were required for through microperforation of the nail plate, for active delivery of the liquid component (MB) of the gel – N_{ad} = 4 ± 1 pulses at h_g = 100 ÷ 500 μm and N_{ad} = 8 ± 1 pulses at h_g = 800 μm. The smallest total number of pulses (N_Σ = 34) necessary for the formation of a microhole in the gel and nail plate followed by active delivery was observed at the gel plate thickness of h_g = 100 μm and agar-agar concentration of C_{aa} = 3%. Thus, at a laser pulse repetition rate of f = 30 Hz, the time required for active laser delivery of a MB through a single microhole when using the gel will be ~1 s. It can be seen that N_Σ is mainly determined by the process of gel plate microperforation.

In Fig. 3 the dependences characterizing the process of microperforation of the gel plate are presented. Figure 3(a) shows the dependence of the number of Er:YLF-laser pulses required for through microperforation of the gel plate (N_{gp}) on the concentration of agar-agar (C_{aa}) at the gel plate thickness h_g = 100, 300, 500 and 800 μm. It can be seen that with

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increasing the gel concentration, in all cases a smaller number of laser pulses is required for its through microperforation. This may be due to an increase in laser light absorption which leads to increase in gel ablation efficiency to as well as with reduction of agar-agar amount which leads to decrease in gel viscosity and strength. Figure 3(b) shows the dependence of the number of Er:YLF-laser pulses required for through microperforation of the gel plate (N_{gp}) on its thickness at C_{aa} = 0.5, 1, 2, and 3%. It is seen that for all concentrations, an increase in the gel plate thickness led to an increase in the number of pulses required for its through microperforation. As it is obvious, that for ablation of more quantity of a substance it is necessary to spend more energy.

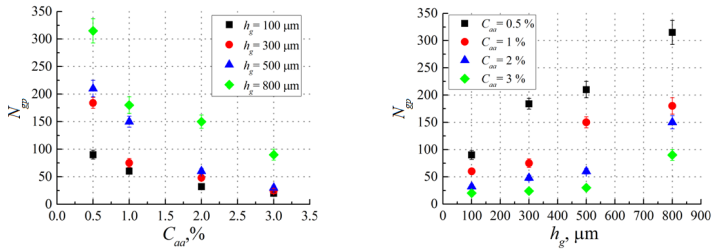

Fig. 3. Dependence of the number of Er:YLF-laser pulses required for through microperforation of the gel (N_{gp}) on the concentration C_{aa} of agar-agar in it (a) and on the gel plate thickness h_g (b) (E = 4.0 mJ).

Figure 4(a) shows the dependence of the delivery rate (V_{MB}) for the gel with C_{aa} = 2% at its different thickness h_g on the laser pulse energy at P = 1.3 kPa. Figure 4(b) shows the dependence of V_{MB} on the gel plate thickness (h_g) at C_{aa} = 2%, E = 4.0mJ and P = 1.3 kPa. The gel plate h_g thickness was varied from 100 to 800 μm. In Fig. 4(c) the dependence of V_{MB} on the magnitude of external pressure (P) applied to the gel for extracting MB from it at C_{aa} = 2%, E = 4.0 mJ and h_g = 500 μm is presented.

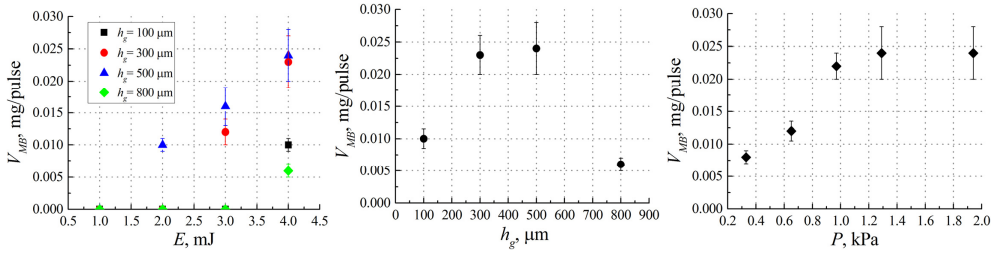

Fig. 4. Dependence of the delivery rate (V_{MB}) for gel with C_{aa} = 2% on laser pulse energy (E) at P = 1.3 kPa (a), on the gel plate thickness (h_g) at E = 4.0 mJ and P = 1.3 kPa (b), on the magnitude of the external pressure (P) applied to the gel for the extraction of the MB from it at E = 4.0 mJ and h_g = 500 μm (c).

With increasing the laser pulse energy, the delivery rate V_{MB} increases for all values of h_g. This may be due to an increase in the volume of laser-produced steam-gas bubbles and the recoil pressure on the evaporation interface, responsible for the delivery process. When the laser pulse energy is E = 1 mJ and the concentration is C_{aa} = 2%, the "initialization" of delivery does not occur. For the "initialization" of drug delivery, at least E = 2 mJ is necessary, but in this case the "initialization" is observed only for h_g = 500 μm. With increasing the laser pulse energy, the delivery rate V_{MB} increases for all values of h_g examined. The maximum value of V_{MB} = 0.024 ± 0.004 mg/pulse was observed at E = 4 mJ and h_g = 500 μm (Fig. 4(a)).

The dependence of the delivery rate V_{MB} on gel plate thickness has an extremum: V_{MB} increases when h_g changing from 100 to 500 μm, and then decreases with increasing h_g from 500 to 800 μm (Fig. 4(b)). This may be due to the peculiarities of MB extraction into a

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microhole in the gel under the action of external pressure P. Most likely, when h_g = 500 μm (for P = 1.3 kPa), the optimum for active laser delivery (at E = 4.0 mJ and C_{aa} = 2%) amount of MB solution is extracted into the microhole. This optimum amount is determined by the nature of hydrodynamic processes occurring in a MB-filled microperforation under the action of Er:YLF-laser pulses.

As the external pressure P increases, the delivery rate V_{MB} increases (Fig. 4(c)). This can be interpreted as follows: the increase in the external pressure P leads to destruction of the walls of a larger number of clusters and, thereby, squeezing out a larger amount of MB into the microhole in the gel plate. The maximum rate (V_{MB}) of MB delivery through a single microhole at C_{aa} = 2% was observed with external pressure applied on the gel $P \geq 1.3$ kPa. At the same time, the dose of MB (D_{MB}) penetrated under the nail plate was 34 ± 3 mg/cm^2, which exceeds the therapeutically sufficient dose [35].

4. Conclusion

In this paper, the possibility of active laser drug (methylene blue) delivery through a single microhole created in a nail plate was experimentally demonstrated. The features of the delivery procedure were the use of the same (Er:YLF) laser to create a microhole and implement the active drug delivery and also the use of agar-agar as a gel matrix for the drug. Active delivery comprises of both mechanical pressure and subsequent impact of laser pulses stimulating hydrodynamic processes. For the first time, the effect of agar-agar concentration in the gel and the thickness of gel plate on the number of Er:YLF-laser pulses required for through microperforation of the gel plate, through microperforation of the nail plate *in vitro* and subsequent active delivery of methylene blue was investigated. The influence of the energy of Er:YLF-laser pulse, the thickness of gel plate, and the external pressure applied to extract the MB solution from the gel on the rate of drug delivery through a single microhole in the nail plate *in vitro* was studied.

It was established that the minimum number of pulses (N_Σ = 34) required for the formation of a microhole in the gel and in the nail plate with subsequent active drug delivery corresponds to the gel plate thickness of h_g = 100 μm, agar-agar concentration C_{aa} = 3%, external pressure P = 1.3 kPa and laser pulse energy E = 4.0 mJ. At laser pulse repetition rate of f = 30 Hz it allows to perform active laser delivery of methylene blue through a single microhole in about 1 s. If an active delivery through an array of microholes is required, it should be noted that with sequential microperforation of the nail plate with subsequent active delivery (by scanning), the time of the procedure depends on speed of scanning and the number of microholes, but for simultaneous (without scanning) receiving an array of microholes, only 1 s will be required for active drug delivery.

Funding

Government of Russian Federation (Grant 08-08).

Acknowledgments

The authors are grateful to ITMO University (Saint Petersburg, Russia) for providing equipment and support to this study. The authors are also grateful to Yuliya V. Semyashkina (ITMO University, Saint Petersburg, Russia) for fruitful discussion of the results of this study.

Disclosures

The authors declare that there are no conflicts of interest related to this article.

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